PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification** [5] :
C12N 1/06, C12M 3/08

A1

(11) **International Publication Number:** **WO 94/13783**

(43) **International Publication Date:** 23 June 1994 (23.06.94)

(21) **International Application Number:** PCT/EP93/03406

(22) **International Filing Date:** 3 December 1993 (03.12.93)

(30) **Priority Data:**
P 42 41 154.8 7 December 1992 (07.12.92) DE

(71) **Applicant** *(for all designated States except US)*: LANCASTER GROUP AG [DE/DE]; Frankfurterstrasse 8, D-65189 Wiesbaden (DE).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: ZASTROW, Leonhard [DE/DE]; Grabenweg 13, D-65205 Wiesbaden-Nordenstadt (DE). STANZL, Klaus [DE/DE]; Am Eschbach 9d, D-56323 Waldesch (DE). RÖDING, Joachim [DE/DE]; Trompeterstrasse 19, D-65207 Wiesbaden (DE). GOLZ, Karin [DE/DE]; Florastrasse 39, D-13137 Berlin (DE).

(74) **Agents:** JUNG, Elisabeth et al.; P.O. Box 14 68, D-80714 München (DE).

(81) **Designated States:** AU, BR, CZ, FI, HU, JP, NO, NZ, PL, RO, SK, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) **Title:** PROCESS FOR DISINTEGRATING CELL DISPERSIONS OR CELL SUSPENSIONS BY MEANS OF ULTRASONICATION FOR THE PURPOSE OF ISOLATING CELL CONSTITUENTS

(57) **Abstract**

The invention relates to a process, which can be carried out continuously, for disintegrating cell material in the form of dispersions or suspensions in water for the purpose of obtaining cell constituents. The invention avoids the use of solid ultrasonication activators and the establishment of a particular geometric form for the acoustic irradiation container by particular parameters with regard to the synotrode angle, the depth of immersion of the synotrode, the ratio of extent of immersion of the synotrode to the acoustic irradiation volume and the ratio of extent of immersion of the synotrode to the solide matter content of the medium to be sonicated. In this process, contents of solid matter within the range from 0.5 to 65 % by weight can be used in the medium.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

Process for disintegrating cell dispersions or cell suspensions by means of ultrasonication for the purpose of isolating cell constituents

Cell constituents, such as enzymes, proteins, vitamins and substances having an antimethodic inflammation-inhibiting or cytostatic effect, are required, for example, in medical, pharmaceutical and cosmetic applications.

DE-C-32 26 016 describes an arrangement with an extrusion homogenizer in which the cells are destroyed by a high pressure gradient and cavitation and turbulence effects in a narrow aperture.

A fundamental disadvantage of these processes and arrangements is that they are very time-consuming and the degree of disintegration they achieve is unsatisfactory.

It is also disadvantageous that in many cases only long-lasting organic compounds can be dealt with. In addition to this, the abovementioned mechanical processes are very energy-consuming and give rise to high installation and running costs, and their efficiency for use with relatively susceptible substances remains limited.

Of the possible disintegration methods in which ultrasonication devices are used, only a few are known from the literature and from the catalogues of manufacturing companies which are additionally limited to laboratory use. These processes are characterized by the known arrangement of an ultrasonication device consisting of an HP generator, an electromechanical converter with an operating tool (synotrode) and a multiplicity of acoustic irradiation vessels which are mostly open and which in addition can be coolable and permit continuous charging with the medium.

Special acoustic irradiation devices (cells) are also known which are coupled directly to an

electromechanical ultrasonication converter (cf., for example, DE-C-20 27 533). It is disadvantageous that, as a result of the necessary configuration as a wavelength-dependent resonator element, no advantageous constructional design of the acoustic irradiation volume is possible and cooling must be dispensed with.

Many forms of synotrode are known from industrial descriptions of inventions which are suitable for acoustic irradiation. A crucial disadvantage of these processes and arrangements is the unsatisfactory degree of disintegration achieved, amounting to at most 60%, occasioned by the fact that no agents favouring the ultrasonication effect are employed and that no allowance is made for the necessary constructional design of an acoustic irradiation volume.

In order, as far as possible, to remedy these deficiencies, DD 284 131 recommends the concomitant use of so-called ultrasonication activators, for example in the form of bodies consisting of a cavitation-resistant and reverberative material, such as hard ceramic. These bodies occupy a relatively large proportion of the volume of the acoustic irradiation space and therefore diminish its receptive capacity for the medium to be sonicated. In addition to this, only media having solid matter concentrations of at most 19% by weight can be treated in practice. A further disadvantage of this known method is that it is necessary to use a spherical acoustic irradiation space in the centre of which the radiation surface of the synotrode is arranged.

The object of the invention was, therefore, to overcome the limitations with regard to the solid matter concentrations of the medium to be sonicated and with regard to the nature of the acoustic irradiation space and the arrangement of the synotrode, and to make available an acoustic irradiation process which permits optimum cell disintegration at solid matter concentrations of up to 65% by weight in a flow-through cell without activating bodies.

This object is achieved by the process having the

features of the main claim and in which, surprisingly, it is no longer necessary for the acoustic irradiation space to be spherical and it is possible to use any desired spatial shape which is favourable for purification.

The process is very expediently carried out at an amplitude within the range from 20 to 70.

The optimum for the synotrode angle in the acoustic irradiation space is 85.3°.

The medium to be sonicated can contain solid matter in concentrations within the range from 0.5 to about 65% by weight.

In practice, performance of the novel process does not present any difficulties, since the cell dispersion or suspension in water is pumped by means of a pump through the cooled flow-through vessel in which the synotrode is arranged with due regard to angle setting and immersion depth, the extent of immersion of the synotrode simultaneously being adjusted, in the manner indicated, to the relevant acoustic irradiation volume.

The invention is explained in more detail by the examples below.

Example 1.

Disintegration of yeasts:

- Baking yeasts
- Brewing yeasts
- Wine-making yeasts
- Special yeasts, e.g. SOD-enriched, etc. (SOD = superoxide dismutase)

Recipe:

23.5% by weight yeast, e.g. baking yeast
10.0% by weight glycerol
5.5% by weight propylene glycol
q.s. distilled water

Preparation:

Preparation temperature: 5 to 7°C

Distilled water is initially introduced into a container. The yeast is dispersed in the water by stirring. The glycerol and the propylene glycol are then added to the suspension.

Disintegration:

The homogeneous yeast suspension is conducted through the flow-through vessel by means of a pump and it is exposed in the vessel to ultrasonication. This entails careful cell disintegration with the isolation of active cell constituents, such as, for example, proteins: e.g. Zn + Cu superoxide dismutase; vitamins, such as, for example, vitamin B complex, A and E.

Parameters:

- Amplitude:	55	
- Synotrode angle:	85.3	°
- Time unit (flow-through speed):	1	1/h
- Total volume of the flow-through container:	550	ml
- Length of synotrode in the vessel:	30	mm
- Proportion of solid matter:	23.5	% by weight
- Extent of disintegration:	95 - 99	%

In this case, the relationship:
length of synotrode:volume:proportion of solid matter
is 1 : 18 : 0.8.

The total length of the synotrode is 50 mm. The ratio of the length of the synotrode in the vessel to its total length is therefore 0.6.

Example 2.

Disintegration of the bark of the Mexican skin tree:

Recipe:

35.0% by weight skin tree, pulverized
5.0% by weight glycerol
5.0% by weight propylene glycol
q.s. distilled water

Preparation:

Preparation temperature: max. 15°C.

Distilled water is initially introduced into a container. The pulverized skin tree material is thoroughly dispersed in the water by stirring. Finally, glycerol and propylene glycol are added.

Disintegration of the skin tree material:

While stirring, the skin tree suspension which has been prepared is pumped into the the flow-through

container and exposed therein to ultrasonication.

Parameters:

- Amplitude: 65
- Synotrode angle: 87.0 °
- Time unit (flow-through speed): 0.5 l/h
- Length of synotrode in the vessel: 33.2 mm
- Volume of the flow-through container: 650 ml
- Proportion of solid matter: 35 % by weight
- Extent of disintegration: 96 % cell constituents having an anti-methodic, cytostatic effect

In this case, the relationship:
length of synotrode:volume:proportion of solid matter
is 1 : 19 : 1.

The total length of the synotrode is 50 mm. The ratio of the length of the synotrode in the vessel to its total length is therefore 0.664.

Example 3.

Disintegration of algae of all kinds:

- e.g. green algae

Recipe:

65.0% by weight algae, e.g. green algae
5.0% by weight glycerol
q.s. distilled water

Preparation:

Preparation temperature: 7 to 10°C

Distilled water is initially introduced into a container. The algae (e.g. green algae) are added while stirring and, following this, the glycerol is distributed homogeneously in the suspension.

Disintegration of the algae:

While stirring, the algal substance which has been prepared is pumped into the flow-through container. The algae are disintegrated by means of ultrasonication in the acoustic irradiation space. The maximum temperature is 10°C.

Parameters:

- Amplitude:	60	
- Synotrode angle:	83.8	°
- Time unit (flow-through speed):	1	l/h
- Length of synotrode in the vessel:	29.5	mm
- Volume of the flow-through container:	100	ml
- Proportion of solid matter:	65	% by weight
- Extent of disintegration:	98.5	%

In this case, the relationship:

length of synotrode:volume:proportion of solid matter

is 1 : 3.4 : 2.2

The total length of the synotrode is 50 mm. The ratio of the length of the synotrode in the vessel to its total length is therefore 0.59.

Example 4:

Disintegration of bacteria:

- e.g. Acinetobacter calcoaceticus

Recipe:

45.0% by weight bacteria, e.g. Acinetobacter calcoaceticus

3.0% by weight glycerol

2.0% by weight propylene glycol

q.s. distilled water

Preparation:

Preparation temperature: 3 to 5°C.

Distilled water is initially introduced into a container. While stirring, glycerol, propylene glycol and bacteria are added consecutively.

Disintegration of the bacteria:

The homogeneous bacterial suspension is pumped into the flow-through vessel and exposed to ultrasonication.

Parameters:

- Amplitude:	45	
- Synotrode angle:	84.9	°
- Time unit (flow-through speed):	1	l/h
- Total volume of the flow-through container:	50	ml
- Length of the synotrode in the vessel:	30.9	mm

- Proportion of solid matter: 45 % by weight
- Extent of disintegration: 99.5 %

In this case, the relationship:

length of synotrode:volume:proportion of solid matter

is 1 : 1.6 : 1.5

The total length of the synotrode is 50 mm. The ratio of the length of the synotrode in the vessel to its total length is therefore 0.618.

Example 5.

Disintegration of seeds and grains:

- e.g. flax seed

Recipe:

0.5% by weight flax seed

10.0% by weight propylene glycol

q.s. distilled water

Preparation:

Preparation temperature: max. 15°C.

The flax seeds are added to the water-propylene glycol mixture while stirring.

Disintegration:

Using a pump, the flax seed suspension is conveyed into the ultrasonication vessel and is disintegrated using the following parameters:

Parameters:

- Amplitude: 55
- Synotrode angle: 80.0 °
- Time unit (flow-through speed): 0.5 l/h
- Total volume of the flow-through container: 100 ml
- Length of synotrode in the vessel: 25 mm
- Proportion of solid matter: 0.5 % by weight
- Extent of disintegration: 85 - 87 %

In this case, the relationship:

length of synotrode:volume:proportion of solid matter

is 1 : 4.35 : 0.02

The total length of the synotrode is 50 mm. The ratio of the length of the synotrode in the vessel to its total length is therefore 0.5.

Patent Claims

1. Process for disintegrating cell dispersions or cell suspensions by means of ultrasonication treatment in an ultrasonication flow-through cell for the purpose of obtaining cell constituents, characterized in that the synotrode projects into the flow-through cell by 1/2 to 2/3 of its length, in that the angle of the synotrode in the acoustic irradation vessel is within the range from 80.5 to 88.5°, in that the ratio of the extent of immersion of the synotrode (in mm) to the acoustic irradiation volume (in ml) is set to a value within the range from 1:1.1 to 1:20 and in that the ratio of the extent of immersion of the synotrode (in mm) to the proportion of solid matter in the medium to be sonicated (in per cent by weight) is within the range from 1:0.02 to 1:2.2.

2. Process according to Claim 1, characterized in that an amplitude within the range from 20 to 70 is used.

3. Process according to Claims 1 and 2, characterized in that the synotrode angle is 85.3°.

4. Process according to Claims 1 to 3, characterized in that the concentration of solid matter in the medium to be sonicated is within the range from 0.5 to 65% by weight.

INTERNATIONAL SEARCH REPORT

Inter .onal Application No
PCT/EP 93/03406

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N1/06 C12M3/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 288 618 (GEN-PROBE INCORPORATED) 2 November 1988 see page 5, line 42 - page 6, line 4 ---	1,2
A	DE,A,21 55 176 (COTTELL, E.C.) 10 May 1972 see claims; figures ---	1
A	CHEMICAL ABSTRACTS, vol. 75, no. 17, 25 October 1971, Columbus, Ohio, US; abstract no. 108502c, HEYSE, K.U. & PIENDL, A. 'Disruption of brewing yeasts by ultrasonics.' page 293 ;column 1 ; see abstract & BRAUWISSENSCHAFT vol. 24, no. 7 , 1971 , GERMANY pages 231 - 238 --- -/--	4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 March 1994	08. 04. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Bevan, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/EP 93/03406

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOTECHNOLOGY AND BIOENGINEERING. vol. 19 , 1977 , NEW YORK US pages 649 - 660 DOULAH, M.S. 'Mechanism of disintegration of biological cells in ultrasonic cavitation.' -----	

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. ional Application No
PCT/EP 93/03406

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0288618	02-11-88	AU-B-	604684	03-01-91
		AU-A-	7040487	24-09-87
		CA-A-	1296604	03-03-92
		JP-B-	5028599	26-04-93
		JP-A-	62236478	16-10-87
		JP-A-	5068553	23-03-93
DE-A-2155176	10-05-72	AU-B-	463704	07-08-75
		AU-A-	3537371	10-05-73
		BE-A-	774982	05-05-72
		CA-A-	967947	20-05-75
		CH-A-	536132	30-04-73
		FR-A-	2113655	23-07-72
		GB-A-	1363934	21-08-74
		NL-A-	7115268	09-05-72
		SE-B-	387056	30-08-76
		US-A-	3715104	06-02-73